

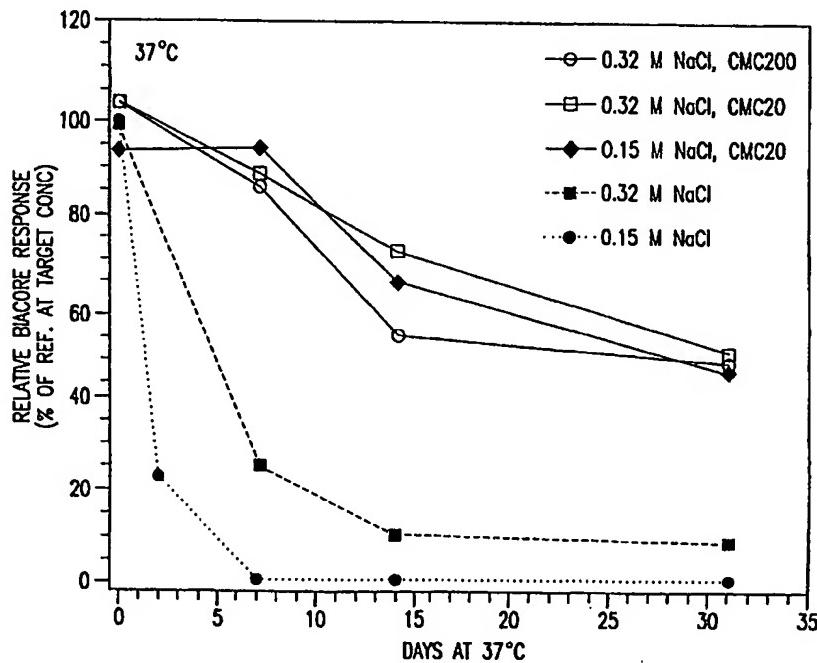


INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : A61K 39/12, 47/00, A61P 31/20	A2	(11) International Publication Number: WO 00/45841
		(43) International Publication Date: 10 August 2000 (10.08.00)

(21) International Application Number: PCT/US00/02463	(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).
(22) International Filing Date: 1 February 2000 (01.02.00)	
(30) Priority Data: 60/118,723 5 February 1999 (05.02.99) US	
(71) Applicant (<i>for all designated States except US</i>): MERCK & CO., INC. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).	
(72) Inventors; and	
(75) Inventors/Applicants (<i>for US only</i>): VOLKIN, David, B. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). SHI, Li [CN/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). MACH, Henryk [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).	
(74) Common Representative: MERCK & CO., INC.; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).	

(54) Title: HUMAN PAPILLOMA VIRUS VACCINE FORMULATIONS



(57) Abstract

New human papilloma virus (HPV) vaccine formulations exhibit enhanced long-term stability. Formulation components can include: virus-like particles (VLPs) absorbed onto aluminum, a salt, non-ionic surfactant, and a buffer. Additional formulations also contain a polymeric polyanionic stabilizer and a salt either in the presence or absence buffering agents and nonionic detergent.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon	KR	Republic of Korea	PL	Poland		
CN	China	KZ	Kazakhstan	PT	Portugal		
CU	Cuba	LC	Saint Lucia	RO	Romania		
CZ	Czech Republic	LI	Liechtenstein	RU	Russian Federation		
DE	Germany	LK	Sri Lanka	SD	Sudan		
DK	Denmark	LR	Liberia	SE	Sweden		
EE	Estonia			SG	Singapore		

HUMAN PAPILLOMAVIRUS VACCINE FORMULATIONS

FIELD OF THE INVENTION

5 This invention related to human papillomavirus (HPV) vaccine formulations which provide enhanced long-term storage stability.

BACKGROUND OF THE INVENTION

Human Papillomavirus (HPV) infects the genital tract and has been
10 associated with various dysplasias, cancers, and other diseases. These diseases are currently targets for vaccine development. and vaccines containing virus-like particles (VLPs) which contain L1 or the combination of L1+L2 proteins are currently in clinical trials.

It has been found, however, that HPV VLPs are not stable during long-
15 term storage, either in solution or when absorbed onto aluminum adjuvant particles.

In order to develop a commercially useful vaccine, a stable formulation is needed.

BRIEF DESCRIPTION OF THE INVENTION

20 This invention relates to human papillomavirus (HPV) vaccine formulations which exhibit long-term stability, the vaccines comprising: a) HPV virus-like particles (VLPs) which are adsorbed on an aluminum adjuvant; b) a salt; c) a buffer which provides for a pH range of the vaccine solution of from about pH 6.0 to about 6.5; and d) a non-ionic surfactant. In another embodiment, the formulation 25 further comprises a polymeric polyanionic stabilizer.

Another embodiment of this invention is a vaccine comprising: a) 10-200 mcg/ml of each HPV VLP type adsorbed onto aluminum; wherein the VLPs are selected from the group consisting of: HPV 6a, HPV 6b, HPV 11, HPV 16, HPV 18, and mixtures thereof; b) 0.15 M NaCl; c) 0.05% carboxymethyl cellulose; and d) 30 optional buffer agents and/or nonionic detergents.

This invention also relates to an improved stable vaccine formulation made by (i) adjusting the ionic strength of the solution with varying concentrations of salts; adjusting and controlling the pH of the solution with particular buffering agents; (iii) adding an non-ionic surfactant; and (iv) adding additional stabilizing excipients in 35 the form of polymeric polyanions.

BRIEF DESCRIPTION OF THE FIGURES

FIGURE 1A is a graph showing the effects of NaCl concentration and
5 added excipients on the stability of HPV16 VLP-aluminum formulations at 2-8°C;
FIGURE 1B shows the results at 15°C.

FIGURE 2 is a graph showing the effects of excipients on the
accelerated stability of HPV16 VLP-aluminum formulations.

10 FIGURE 3 is a bar graph showing the effects of polyanion excipients
on the accelerated stability of HPV16 VLP-aluminum formulations.

FIGURE 4 is a bar graph illustrating the effect of carboxy-methyl
cellulose (CMC) concentration on the accelerated stability of HPV16VLP-aluminum
formulations.

15 FIGURE 5 is a graph showing the effects of carboxymethyl cellulose
(CMC) molecular weight and NaCl concentration on the accelerated stability of
HPV16 VLP-aluminum formulations.

Under physiological solution conditions of salt and pH, the virus like
particles (VLPs) of Human Papillomavirus (HPV) L1 protein are not stable during
20 long term storage either in solution or after adsorption to aluminum adjuvant at 2-8°C.
Thus this invention relates to new formulations of both aluminum adjuvanted and
non-aluminum adjuvanted HPV VLP vaccines with improved storage stability. The
formulations may be in aqueous solution. In general, the HPV is absorbed onto
aluminum adjuvant.

25 In accordance with this invention, any type of HPV VLPs may be used
as the antigenic portion of the vaccine formulation. The VLPs may contain only L1
protein, or may be made of both L1 and L2 protein. The proteins may be of a wild-
type amino acid composition, or they may contain mutations. VLPs containing only
L1 protein which is of a wild-type amino acid composition are preferred.

30 The HPVs which are particularly preferred are those associated with
disease, including but not limited to: HPV 6a, HPV 6b, HPV11, HPV16, and HPV18.
In addition, the formulations of this invention are suited for combinations of HPV
types, including multivalent vaccines containing a plurality of HPV antigens, such as
a combination of HPV 6, 11, 16 and 18. It is preferred that the VLPs be made by
35 recombinant techniques, as is known in the art. It is particularly preferred that the

host cell used to make the VLPs is a yeast cell, although other cell types, such as bacterial, insect and mammalian cells are known and currently used as hosts.

Generally, the concentration of HPV VLPs which are absorbed onto aluminum is from about 10-200 mcg/ml for each HPV VLP type. This may be adjusted, depending on such factors as antigenicity of the particular type of HPV, and the presence of multiple types of HPVs in a "cocktail"-type of vaccine.

One embodiment of this invention is a vaccine comprising a) HPV virus-like particles (VLPs) which are adsorbed on an aluminum adjuvant; b) a salt; c) a buffer which provides for a pH range of the vaccine solution of from about pH 6.0 to about 6.5; and d) a non-ionic surfactant. A further embodiment of this invention further comprises a polymeric polyanion stabilizer. Another embodiment of this invention is a formulation which omits the higher salt concentration, and comprises HPV VLPs adsorbed onto aluminum, a physiological concentration of salt (about 0.15M), and a polymeric polyanionic stabilizer in the presence or absence of buffer agents and nonionic detergents.

Salts

The ionic strength of the solution is maintained by the presence of salts. Almost any salt which can contribute to the control of the ionic strength may be used. Preferred salts which can be used to adjust ionic strength are: any physiologically acceptable salt, such as NaCl, KCl, Na₂SO₄, (NH₄)₂SO₄, sodium phosphate and sodium citrate. Particularly preferred salts are: NaCl, KCl, and Na₂SO₄. It has been found that increasing ionic strength dramatically enhances the stability of HPV VLPs against heat induced aggregation. For example, the stability of HPV VLP protein solution was analyzed for aggregate formation as a function of temperature by using an UV spectrophotometer (turbidity assay for cloud point determination). The cloud point data indicate that increasing ionic strength (using 0.15M to 1M NaCl) dramatically enhances the stability of HPV VLPs in solution against heat-induced aggregation with the temperature of the initiation of turbidity formation being raised approximately 7°C.

The salts should be present in concentrations of from about 0.10M to 1M. However, very high concentrations are not preferred due to the practical limitations of parental injection of high salt concentrations. Instead, more moderate salt concentrations, such as more physiological concentrations of about 0.15M to about 0.5M with 0.15M-0.32M NaCl are preferred.

Buffers

- Some formulations of this invention also contain a buffer to maintain the pH range so that the vaccine is in the non-irritating pH range with optimal HPV VLP stability. The effect of pH on the stability of HPVs both in solution and adsorbed to aluminum formulation were also investigated in accordance with this invention. Results indicate that HPV VLPs are stable only within a relatively small range of pH 5.5-7.0, and that the preferred pH range is 6.0-6.5, and particularly 6.2 as measured by *in vitro* antigenicity.
- The storage stability of HPV-aluminum formulations was further tested with the addition of a buffer and non-ionic surfactant. Better pH control of the aluminum adjuvanted HPV VLP vaccine during storage was observed when either histidine or imidazole was added as a buffer agent. In general, the concentration of the buffer should range from about 2 mM to about 100 mM, with 5 mM to about 20 mM being preferred, and 10 mM being another preferred concentration. Phosphate-containing buffers are generally not preferred, as they may interact with aluminum adjuvants. The interaction of phosphate buffer ions with aluminum adjuvant as well as the non interaction of histidine and imidazole buffers with aluminum adjuvant was demonstrated by zeta potential measurements of the surface charge of the aluminum adjuvant.

Non-ionic surfactant

- A further component in some of formulation of this invention is a non-ionic surfactant. The surfactant may be selected from the group consisting of: polyoxyethylene sorbital fatty acid esters (Polysorbates) such as Polysorbate 80 (e.g., TWEEN 80[®]), Polysorbate 20, (e.g., TWEEN 20[®]) polyoxyethylene alkyl ethers (e.g. Brij 35[®], and Brij 58[®]), as well as others, including Triton X-100[®], Triton X-114[®], NP-40[®], Span 85 and the Pluronic series of non-ionic surfactants (e.g. Pluronic 121), with Polysorbate 80 being particularly preferred. The surfactant is generally present in a concentration of from about 0.0005% to about 0.5% (wt/vol). Polysorbate 80 was found to protect HPV VLP-aluminum from inactivation during simulated shipping stress (i.e., shaking or stirring). For Polysorbate 80, a preferred concentration is about 0.01%.

Polymeric polyanion stabilizer

- A further significant enhancement of the stability of the HPV-VLP formulation may also accomplished by the addition of polyanionic, polymeric

excipients, such as a stabilizer. As used throughout the specification and claims, the term "polyanionic polymer" is meant to refer to compounds which have either a single long chain, or those with multiple cross linked chains; either type possessing multiple negative charges along the chain(s) when in solution. Examples of polyanionic polymers include: proteins, polyanions, peptides and poly-nucleic acids. Specific stabilizers may be selected from the group consisting of:

- 5 carboxymethyl cellulose (particularly 10-800cps),
- heparin (6-30 kDa),
- poly-amino acids (2-100 kDa) such as poly(Glu), Poly(Asp), and
- 10 Poly(Glu, Phe),
- oxidized glutathione [Glu-Cys-Gly]₂ (613 Da),
- poly-nucleotides such as polycytidylic acid (200-700 kDa), and
- polyadenylic acid(200-700 kDa),
- RNA,
- 15 DNA, and
- serum albumins.

The concentration of the stabilizer, when present, is from about 0.01% to about 0.5%, particularly about 0.05-0.1% (by weight), although the addition of even a ten fold lower amount of polyanionic excipients (for example, 0.01% albumin, 20 DNA or heparin) still provides enhanced stability to HPV VLP-aluminum formulations, although the stabilizing effect is relatively less significant perhaps due to the lower concentrations. As described in more detail in the Examples, polyanions provided a dramatic stabilization of HPV-aluminum formulations. The stabilizing mechanisms of these classes of excipients may vary from directly binding to HPV 25 VLP molecule (such as occurs with DNA), inhibiting HPV VLP or HPV VLP-aluminum adsorption on surfaces, increasing solution viscosity, neutralizing surface charge, reformation of HPV VLP disulfide bonds or interfering with amino acid side chain oxidation, and/or increasing the conformational rigidity of HPV VLPs. It is important to note that HPV VLP L1 protein contains a polyanion binding site of 30 multiple positively charged amino acids in the C-terminal region of the protein.

These polyanions were further tested under accelerated stability study conditions at 37°C. Polyanion containing formulations of HPV-aluminum retained about 80% of *in vitro* antigenicity while the control HPV formulation (without addition of polyanions) had almost no *in vitro* antigenicity after one week of 35 incubation at 37°C. The same set of samples after two weeks of incubation at 37°C

show no significant difference from the one-week data indicating the strong stabilizing effect of these polyanionic excipients.

The polyanion stabilization effect was also investigated for HPV VLPs in solution under accelerated conditions at 37°C. The data indicate that for 5 polynucleotide polyanions, poly(A) or poly(C), a concentration of 0.001% (by weight or 10 mcg/ml) is required for HPV16 VLP (at 80 mcg/ml protein) to maintain maximum stability against heat induced inactivation as measured by *in vitro* antigenicity. Sedimentation and UV analysis indicates that polyanions bind to the VLP directly.

10 In addition, HPV VLP adsorption to aluminum adjuvant may be inhibited by prebinding of polyanions to the HPV VLP. For example, carboxymethyl cellulose can inhibit HPV VLP binding to aluminum at certain concentrations of the polyanion. The addition of carboxymethyl cellulose after HPV VLP adsorption to aluminum adjuvant, however, results in virtually no detectable release of HPV VLP 15 from aluminum while providing a dramatic stability enhancement in a concentration dependent manner.

Especially preferred among the polyanions (0.001 - 0.25% by weight) is carboxymethyl cellulose, (10-800 cps) with an approximate typical molecular weight of 50,000-700,000 Da, with lower viscosity 10-200 cps carboxymethyl 20 cellulose being especially preferred.

Preferred formulations of this invention include the following:

I. a) 10-200 mcg/ml of each HPV VLP type absorbed onto aluminum, wherein the VLPs are selected from the group consisting of: HPV 6a, HPV 6b, HPV 25 11, HPV 16, HPV 18, and mixtures thereof; b) 0.32 M NaCl; c) 10 mM histidine buffer, pH 6.2; and d) 0.01% Polysorbate 80.

II. a) 10-200 mcg/ml of each HPV VLP type absorbed onto aluminum, wherein the VLPs are selected from the group consisting of: HPV 6a, HPV 6b, HPV 30 11, HPV 16, HPV 18, and mixtures thereof; b) 0.32 M NaCl; c) 10 mM histidine buffer, pH 6.2; d) 0.01% Polysorbate 80, and 0.05% carboxymethyl cellulose.

III. a) 10-200 mcg/ml of each HPV VLP type adsorbed onto aluminum, wherein the VLPs are selected from the group consisting of: HPV 6a, HPV 6b, HPV 35 11, HPV 16, HPV 18, and mixtures thereof; b) 0.15 M NaCl; and c) 0.05%

carboxymethyl cellulose; and d) optionally 10 mM histidine, pH 6.2 and 0.01% polysorbate 80.

The following non-limiting Examples are presented to better illustrate
5 the invention.

EXAMPLE 1
General Methods

10 A frozen solution of yeast derived recombinant L1 protein HPV16 VLP (with greater than 95% purity) at a protein concentration of 870 mcg/ml in 0.5M NaCl, 0.003% polysorbate 80, pH of approximately 6.2 was used for most of these experiments. Aluminum adjuvant was manufactured at Merck. Excipients were purchased commercially. All the vaccine formulations were prepared as follows: The
15 HPV VLP solution with or without dilution was added to aluminum adjuvant for HPV VLP alum adsorption, then the excipients were added. All final desired concentrations for all excipients, HPV VLP and aluminum adjuvant in the formulation could be approached by either direct mixing with designed volume, or by adjusting with a settle/decant process.

20 In vitro antigenicity assays.

To release HPV from the aluminum adjuvant, an aluminum dissolution method was developed which included dilution of HPV-aluminum formulation into a high salt solution containing citrate and polysorbate 80. The HPV VLP samples from
25 the aluminum dissolution method are directly subjected to an *in vitro* antigenicity assay using Biacore analysis (utilizing an HPV VLP type specific neutralizing antibody). The HPV VLPs samples from the aluminum adjuvanted stability studies are directly compared to a frozen stock solution of the same HPV VLP to determine *in vitro* antigenicity.

30 pH measurement.

The pH measurements of HPV-aluminum formulations were performed at ambient temperature using an Orion pH meter of model 420A. All samples incubated at various temperatures were equilibrated at room temperature for
35 30 minutes before pH determination. The pH meter was calibrated manually using

two standard buffers that bracket the expected sample pH range, with a correlation coefficient between 95%-100%. Temperature effect on the pH of histidine buffered solutions was determined by varying temperature gradually from 4°C to 37°C.

5 Protein concentration determination (UV spectroscopy)

The protein concentration of HPV in both bulk solutions and formulated samples (after aluminum dissolution) were determined by UV absorbance spectra measurement at ambient temperature using a HP 8452A Diode Array spectrophotometer and a cuvette with a path length of 1 cm. The sample volumes 10 used were approximately 200 to 250 microliters. The protein concentration was calculated using a multicomponent second derivative analysis technique developed for use with HPV VLPs.

Other analyses

15 Sedimentation velocity experiments were performed using Beckman XL1 analytical ultracentrifuge.

Turbidity assays were carried out using a HP 8452A Diode Array spectrophotometer equipped with a HP 845X UV-Visible system software and a temperature control system. The light scattering of the solutions were followed at 20 320-350 nm under the kinetic mode of the program by increasing the temperature from about 25°C to 80°C.

Zeta potential of aluminum particles and HPV VLPs were determined using a Malvern Zetasizer 3000 System with varying the solution pH from 4 to 9 or 25 the excipient concentrations.

Accelerated and Real Time Stability Studies.

HPV-aluminum formulation stability studies were carried out under both accelerated and real time conditions. The temperature of accelerated stability 30 studies varied from 15°C to 37°C. The temperature of real time stability studies was at 2-8°C. These temperature ranges were chosen based on the fact that HPV VLP inactivation rate is very sensitive to temperature. Previous conformational integrity data via biophysical measurements have shown that increasing the temperature to above 40-45°C induces significant conformational changes in the HPV VLP in 35 solution, a condition which needs to be avoided in accelerated stability studies.

EXAMPLE 2

Effects of salt and excipients on stability The effects of NaCl concentration and added excipients on the stability of HPV16 VLP-aluminum formulations at 2-8°C was investigated. Formulations of 160 mcg/ml HPV16 VLP on 450 mcg/ml aluminum adjuvant in different NaCl concentration solution with/without the addition of 0.01% Polysorbate 80, 10 mM histidine were incubated at 2-8°C. The *in vitro* antigenicity of the formulations was then assayed after different incubation times by Biacore analysis. Results are shown in FIGURE 1A. The experiment was then repeated for incubation at 15°C. FIGURE 1B shows the results when incubated at 15°C. In both graphs, closed squares are the points for 0.15M NaCl; open squares are the points for 0.30M NaCl; closed circles are for 0.30 M NaCl, 0.01% Polysorbate 80, and 10 mM histidine. The data indicate that increasing salt concentration enhances the vaccine stability and the addition of buffer and polysorbate further enhances the stability.

15

EXAMPLE 3

Effects of stabilizers The effects of stabilizing excipients on the accelerated stability of HPV16 VLP-aluminum formulations was investigated. Formulations of 200 mcg/ml HPV16 VLP on 450 mcg/ml aluminum adjuvant with/without the addition of different stabilizing excipients were incubated at pH 6.2 and 25°C. The *in vitro* antigenicity of the formulations was then assayed after different incubation times by Biacore analysis. The formulation compositions are shown in FIGURE 2: closed circles are for 0.3M NaCl + 0.1% polyglutamic acid (poly-Glu); crossed squares are for 0.3M NaCl + 0.1% polyadenylic acid (poly-A); open squares are for 0.3M NaCl + 10 mM histidine + 0.01% polysorbate 80; open triangles are for 0.3M NaCl; and open circles are for 0.15M NaCl. The data indicate that at 25°C, increasing salt concentration from 0.15 to 0.3 M and adding buffer and polysorbate enhances the stability of the formulation. The addition of a polyanion, however, more dramatically enhances the stability of aluminum adsorbed HPV VLPs against heat induced loss of *in vitro* antigenicity.

EXAMPLE 4

Effects of polyanions on stability The effects of polyanion excipients on the accelerated stability of HPV16 VLP-aluminum formulations was investigated in more

detail. Formulations of 160 mcg/ml HPV16 VLP on 450 mcg/ml aluminum adjuvant in 0.3 M NaCl, 0.01% Polysorbate 80, 10 mM Histidine, pH 6.2 with/without the addition of a polyanion excipient were incubated at 37°C for 1 week. The *in vitro* antigenicity of the formulations was then assayed by Biacore analysis. The kinds and 5 concentrations of polyanion excipients added are as shown in FIGURE 3, from left to right: control; 0.1% poly Adenylic acid (Poly A); 0.1% poly Cytidylic acid (poly C); 0.1% poly Aspartic acid (poly Asp) (5-15K); 0.1% poly Glutamic acid (poly-Glu) (5-15K); 0.1% poly Glutamic acid (poly-Glu) (50-100K); 0.1% bovine serum albumin (BSA); 0.1% poly Glutamic acid-Phenylalanine (poly Glu, Phe); 0.1% heparin; 0.25% 10 heparin; 2 mM oxidized-Glutathione; 0.1% carboxymethylcellulose (CMC) (200 cps); 0.1% carboxymethyl cellulose (CMC) (800 cps).

It can be seen that all these polymeric polyanions have the ability to significantly stabilize HPV16 VLP-aluminum formulations as seen by the maintenance of *in vitro* antigenicity as measured by Biacore.

15

EXAMPLE 5

The effect of carboxymethyl cellulose The effect of carboxymethyl cellulose (CMC) concentration on the accelerated stability of HPV16 VLP-aluminum formulations was examined. Formulations of 160 mcg/ml HPV16 on 450 mcg/ml aluminum adjuvant in 0.3 M NaCl, 0.01% polysorbate 80, 10 mM Histidine, pH 6.2 with/without the addition of different amount of CMC (200 cps) were incubated at 37°C for 1 week. The *in vitro* antigenicity of the formulations was then assayed by Biacore analysis. Results are shown in FIGURE 4 for 0, 0.005%, 0.01%, 0.05%, 0.1% and 0.2% CMC. The data indicate that the stability enhancement is a function 20 of added CMC concentration. *In vitro* antigenicity is retained in the presence of at least 0.05% CMC.

EXAMPLE 6

Effects of size of CMC The effects of carboxymethyl cellulose (CMC) molecular weight and NaCl concentration on the accelerated stability of HPV16 VLP-aluminum formulations was examined. Formulations of 160 mcg/ml HPV16 VLP on 450 mcg/ml aluminum adjuvant in 10 mM Histidine, pH 6.2, 0.01% polysorbate 80 with/without the addition of CMC were incubated at 37°C. The *in vitro* antigenicity of the formulations was then assayed after different incubation times by Biacore 30 analysis. The CMC concentrations was 0.05%, and the CMC molecular weight is 35

either 10-20 cps or 200 cps level. Results are given in FIGURE 5: open circle is for 0.32M NaCl, CMC 200 cps; open square with solid line is for 0.32 M NaCl, CMC 10-20 cps; closed diamond is for 0.15M NaCl, CMC 10-20 cps; open square with dotted line is for 0.32 M NaCl; and closed circle is for 0.15M NaCl. The data indicate the
5 CMC with smaller molecular weight (about 10-20 cps) basically provides similar stability enhancement to HPV VLP-aluminum formulations with CMC 200 cps and that the presence of CMC allows the vaccine preparation to be formulated at a physiological salt concentration (0.15 M NaCl).

WHAT IS CLAIMED IS:

1. A human papillomavirus (HPV) vaccine formulation comprising:
 - 5 a) HPV virus-like particles (VLPs) which are adsorbed on an aluminum adjuvant;
 - b) a salt;
 - c) a buffer which provides for a pH range of the vaccine solution of from about pH 6.0 to about 6.5; and
 - 10 d) a non-ionic surfactant.
2. A vaccine according to Claim 1 wherein the salt is selected from the group consisting of NaCl, KCl, and Na₂SO₄, (NH₄)₂SO₄, and sodium phosphate and sodium citrate.
15
3. A vaccine according to Claim 2 wherein the salt is present in a concentration of from about 0.10M to about 0.5M.
4. A vaccine according to Claim 3 wherein the salt is 0.32M
20 NaCl.
5. A vaccine according to Claim 1 wherein the buffer is histidine or imidazole.
- 25 6. A vaccine according to Claim 5 wherein the buffer is present in a concentration of about 2 mM to about 100 mM.
7. A vaccine according to Claim 6 wherein the buffer is 10 mM histidine, pH 6.2.
30
8. A vaccine according to Claim 1 wherein the non-ionic surfactant is selected from the group consisting of: Polysorbate 20, Polysorbate 80, Polysorbate 20, NP-40®, Triton X-100®, Triton X-114®, Span 85, Brij 35®, Brij 58®.
35

9. A vaccine according to Claim 8 wherein the surfactant is present in a concentration of from about 0.0005% to about 0.5% (wt/vol).

10. A vaccine formulation according to Claim 1 comprising:
- 5 a) 10-200 mcg/ml of each HPV VLP type absorbed onto aluminum, wherein the VLPs are selected from the group consisting of: HPV 6a, HPV 6b, HPV 11, HPV 16, HPV 18, and mixtures thereof;
- 10 b) 0.32 M NaCl;
- c) 10 mM histidine buffer, pH 6.2; and
- d) 0.01% Polysorbate 80.

11. A vaccine formulation according to Claim 1 which is in an aqueous solution.

15 12. A vaccine according to Claim 1 further comprising a polymeric polyanionic stabilizer.

20 13. A vaccine according to Claim 8 wherein the stabilizer is selected from the group consisting of: carboxymethyl cellulose (CMC), heparin, Poly(Glu), Poly(Asp), Poly (Glu, Phe), oxidized glutathione, polycytidylic acid, polyadenylic acid, RNA, DNA, and serum albumins.

25 14. A vaccine according to Claim 13 wherein the stabilizer is CMC.

15. A vaccine according to Claim 13 wherein the concentration of the stabilizer is from about 0.01% to about 0.5% (by weight/volume).

30 16. A vaccine according to Claim 15 which is in an aqueous solution.

17. An HPV vaccine formulation comprising:

35 a) 10-200 mcg/ml of each HPV VLP type adsorbed onto aluminum; wherein the VLPs are selected from the group consisting of: HPV 6a, HPV 6b, HPV 11, HPV 16, HPV 18, and mixtures thereof;

- b) 0.32 M NaCl;
- c) 10 mM histidine buffer, pH 6.2
- d) 0.01% Polysorbate 80; and
- e) 0.05% carboxymethyl cellulose.

5

18. A human papillomavirus (HPV) vaccine comprising:

- a) HPV virus-like particles (VLPs) which are adsorbed onto aluminum;
- b) a salt
- c) a polymeric polyanionic stabilizer;
- d) optional buffer and/or non-ionic detergent.

10

19. A vaccine according to Claim 18 wherein the salt is selected from the group consisting of NaCl, KCl, and Na₂SO₄, (NH₄)₂SO₄, and sodium phosphate and sodium citrate.

15

20. A vaccine according to Claim 19 wherein the salt is present in a concentration of from about 0.1M to about 0.5M.

21. A vaccine according to Claim 20 wherein the salt is 0.15M
20 NaCl.

22. A vaccine according to Claim 18 wherein the polymeric polyanionic stabilizer is selected from the group consisting of: carboxymethyl cellulose (CMC), heparin, Poly(Glu), Poly(Asp), Poly(Glu, Phe), oxidized 25 glutathione, polycytidylic acid, polyadenylic acid, RNA, DNA, and serum albumin.

23. A vaccine according to Claim 22 wherein the stabilizer is carboxymethylcellulose (CMC).

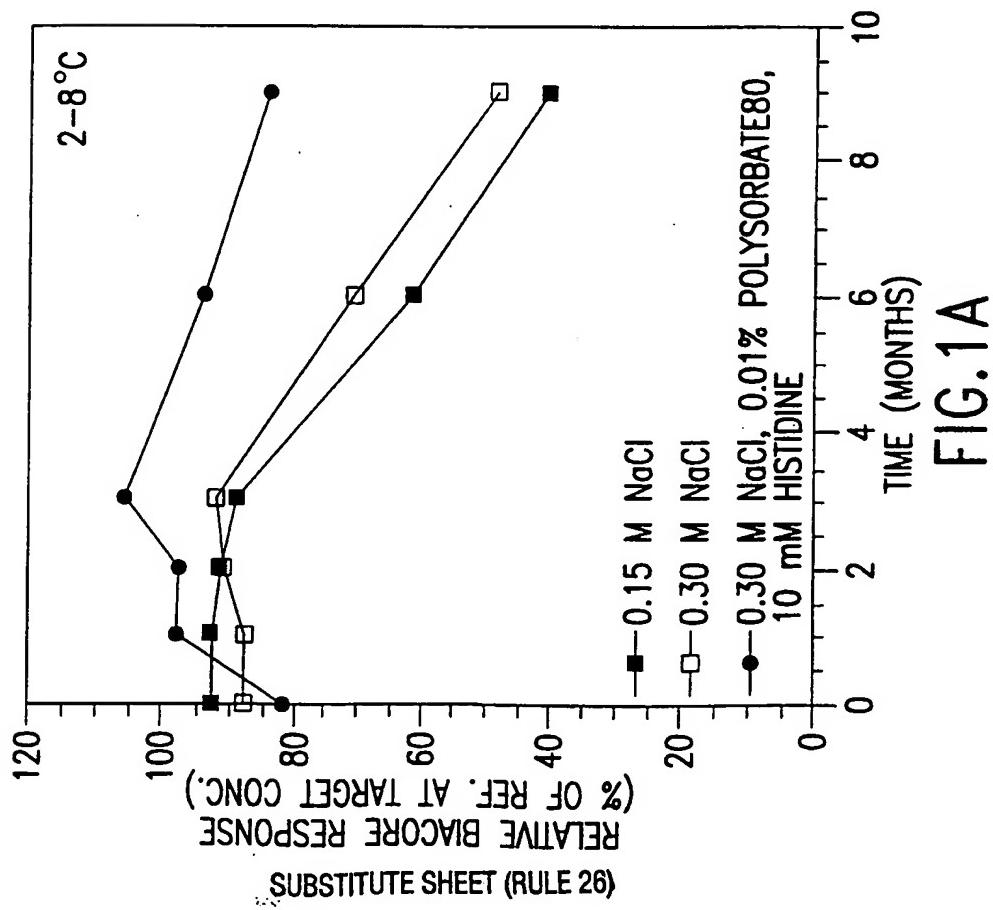
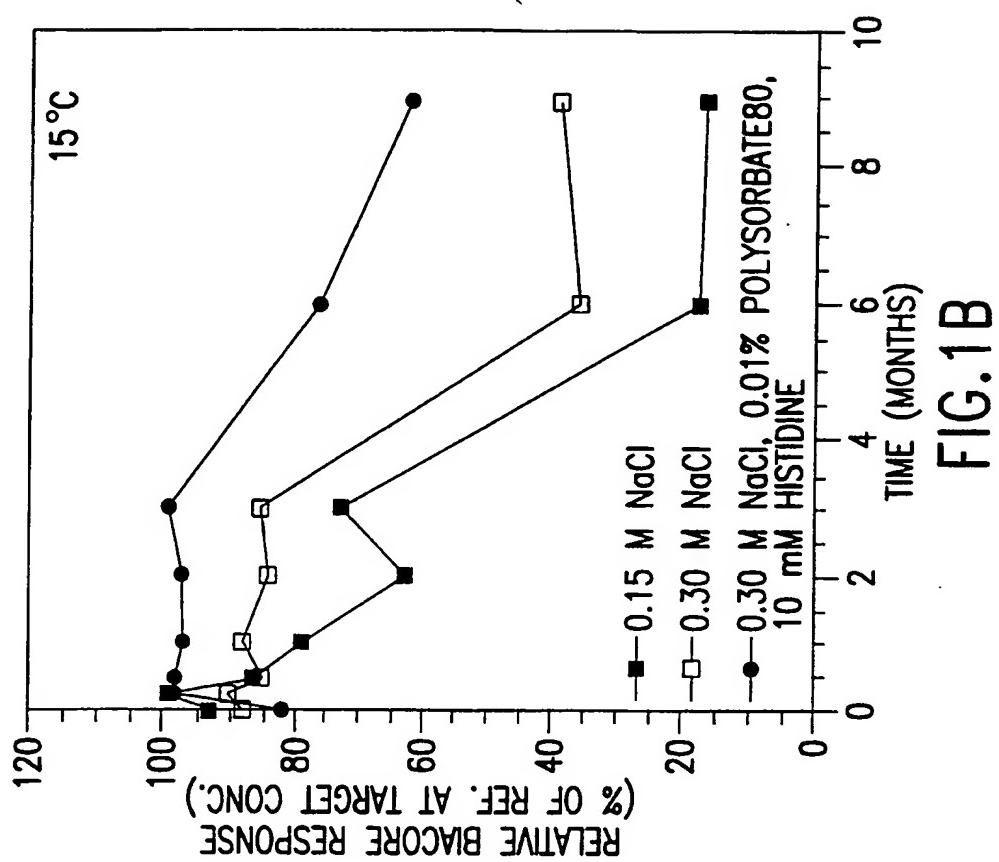
30 24. A vaccine according to Claim 23 wherein the concentration of the stabilizer is from about 0.001% to about 0.5%.

25. A vaccine comprising:

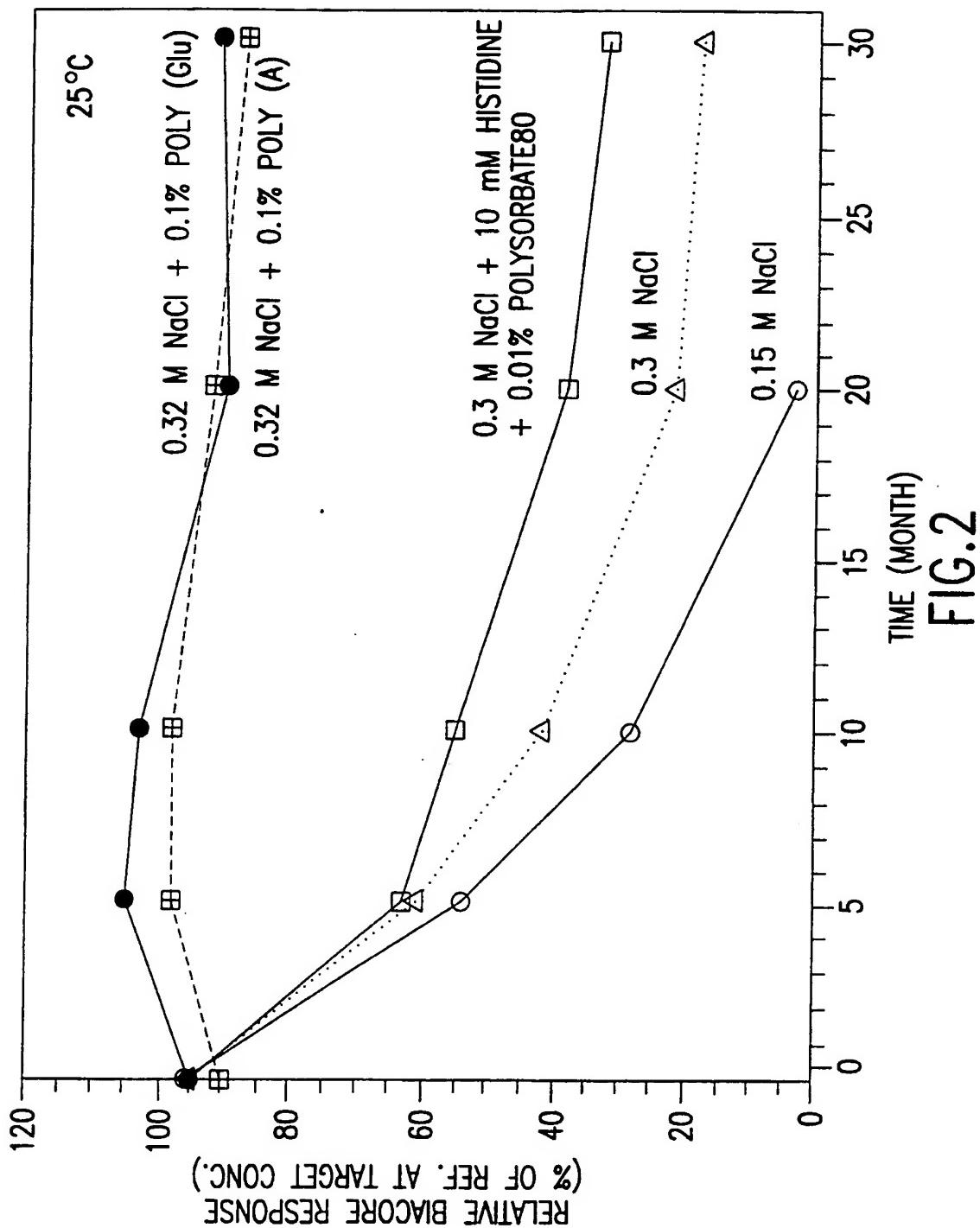
- a) 10-200 mcg/ml of each HPV VLP type adsorbed onto aluminum; wherein the VLPs are selected from the group consisting of: HPV 6a, HPV 6b, HPV 11, HPV 16, HPV 18, and mixtures thereof;
- 5 b) 0.15 M NaCl
- c) 0.05% carboxymethyl cellulose; and
- d) optional buffer and/or non-ionic detergent.
26. A vaccine according to Claim 25 which is in an aqueous solution.

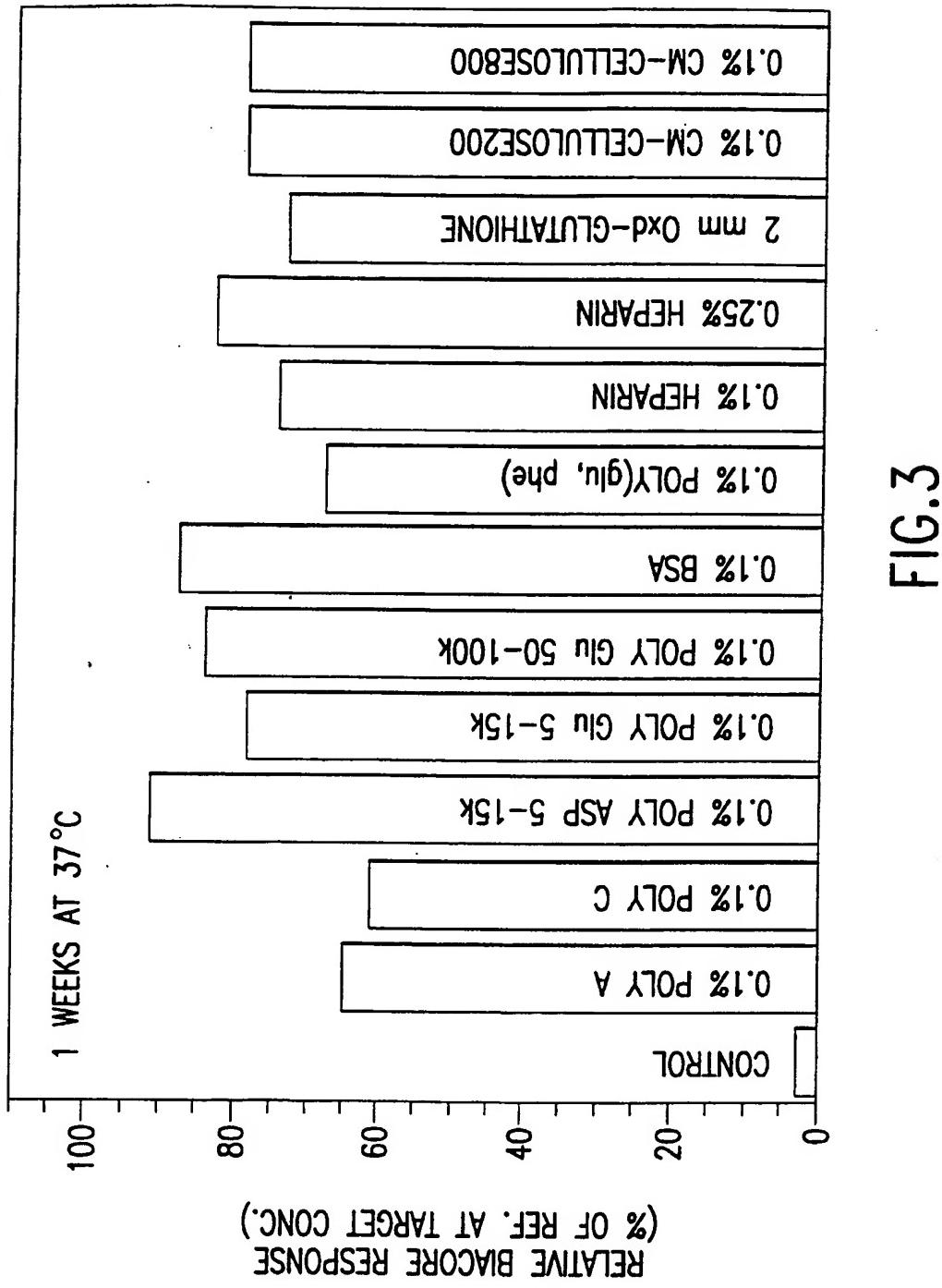
10

1/5



SUBSTITUTE SHEET (RULE 26)





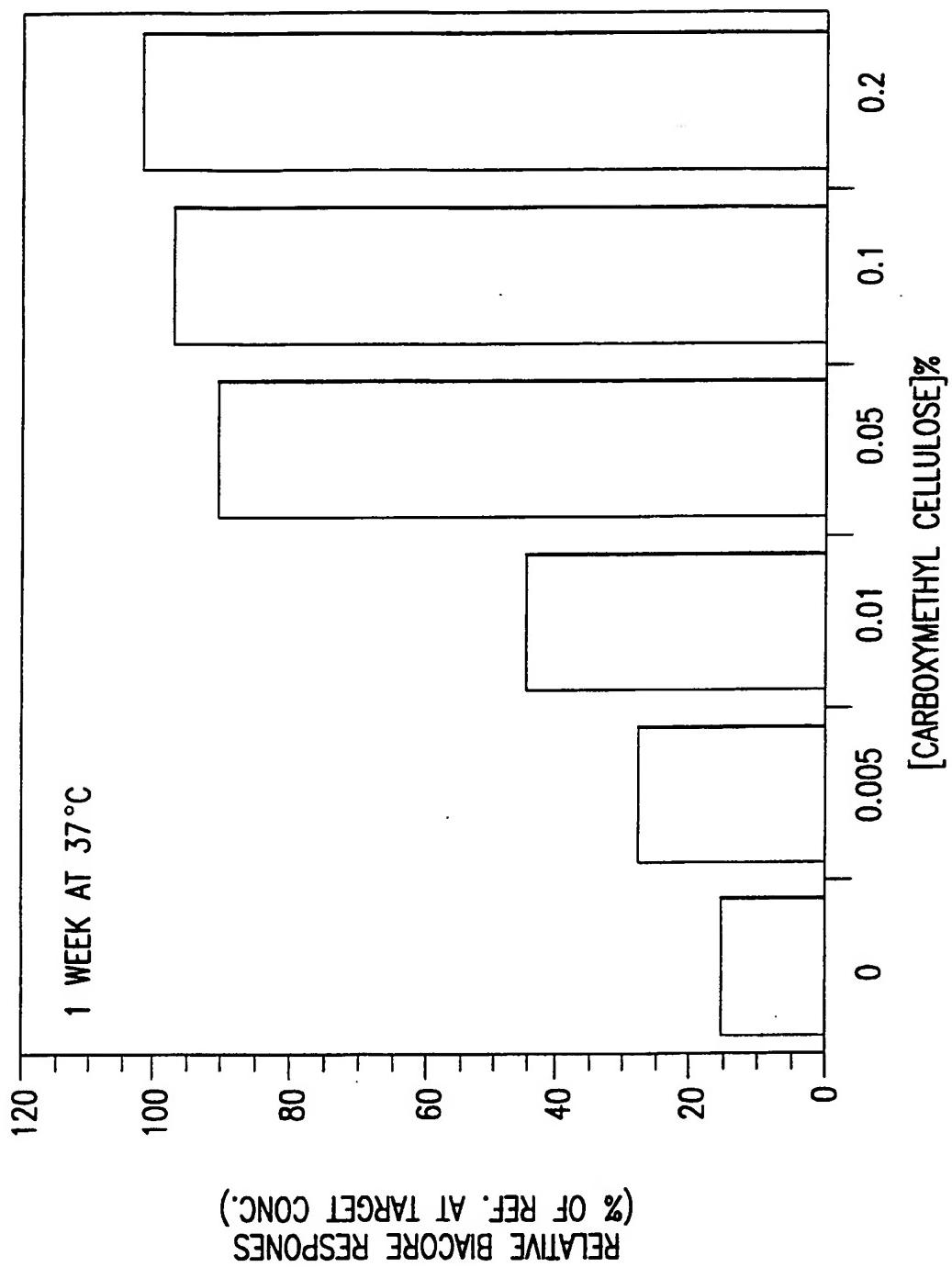
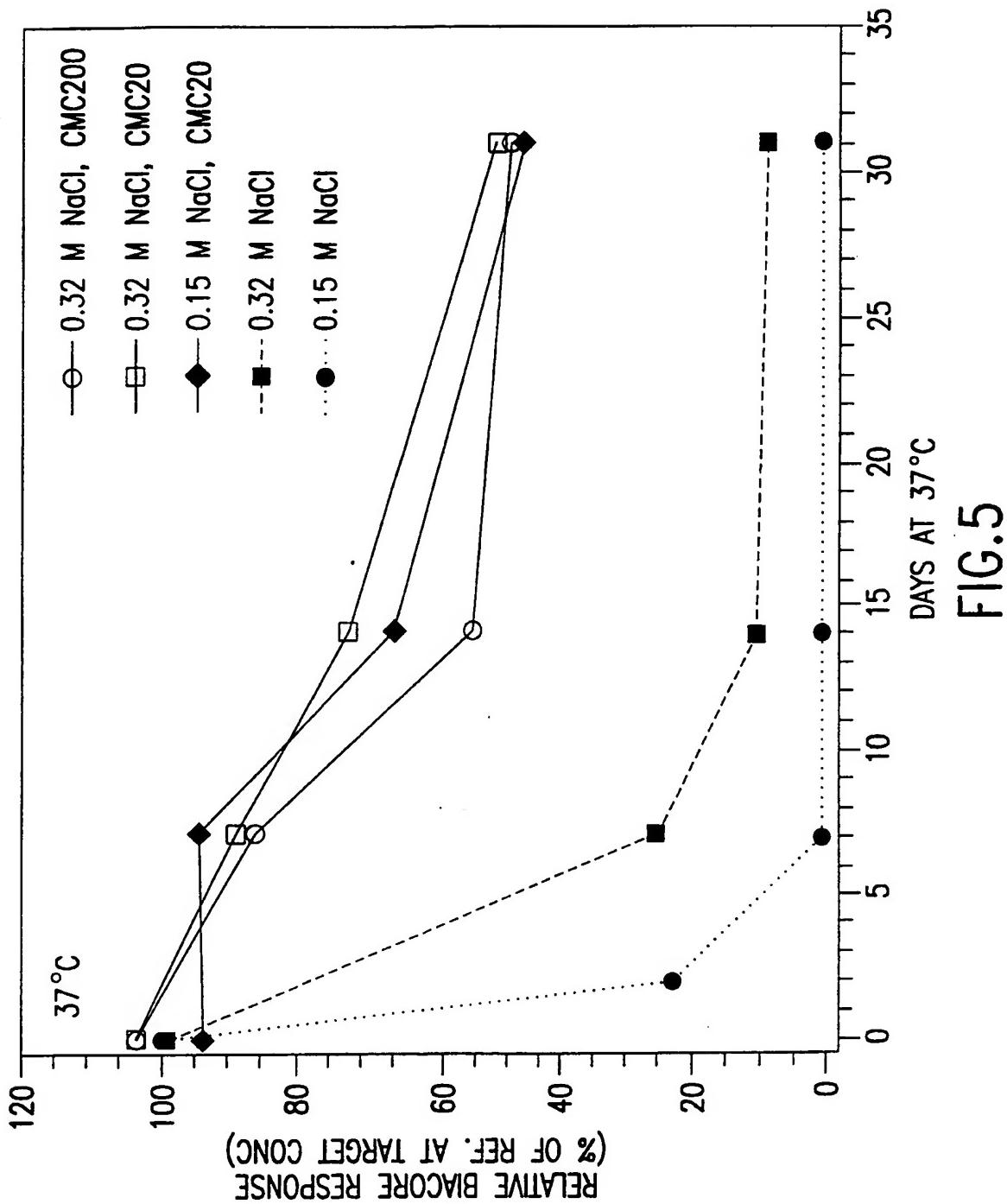


FIG. 4



SUBSTITUTE SHEET (RULE 26)

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
10 August 2000 (10.08.2000)

PCT

(10) International Publication Number
WO 00/45841 A3

(51) International Patent Classification⁷: A61K 39/12, 47/00, A61P 31/20

(81) Designated States (*national*): AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(21) International Application Number: PCT/US00/02463

(22) International Filing Date: 1 February 2000 (01.02.2000)

(25) Filing Language: English

(84) Designated States (*regional*): ARPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

(26) Publication Language: English

(30) Priority Data:
60/118,723 5 February 1999 (05.02.1999) US

Published:

— With international search report.

(72) Inventors; and

(75) Inventors/Applicants (*for US only*): VOLKIN, David, B. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). SHI, Li [CN/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). MACH, Henryk [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).

(88) Date of publication of the international search report:
14 December 2000

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(74) Common Representative: MERCK & CO., INC.; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).

A3
WO 00/45841 A3

(54) Title: HUMAN PAPILLOMA VIRUS VACCINE FORMULATIONS

(57) Abstract: New human papilloma virus (HPV) vaccine formulations exhibit enhanced long-term stability. Formulation components can include: virus-like particles (VLPs) absorbed onto aluminum, a salt, non-ionic surfactant, and a buffer. Additional formulations also contain a polymeric polyanionic stabilizer and a salt either in the presence or absence buffering agents and non-ionic detergent.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/02463

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 7 A61K39/12 A61K47/00 A61P31/20

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
 IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

BIOSIS, EPO-Internal, MEDLINE, CANCERLIT, LIFESCIENCES, CHEM ABS Data, EMBASE, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 44944 A (SANYAL GAUTUM ;MERCK & CO INC (US); SHI LI (US); VOLKIN DAVID B (U) 15 October 1998 (1998-10-15) abstract	1-3,8,9
Y	page 5, line 1 -page 7, line 24 -----	5-7,10, 11,19,20
X	WO 95 31532 A (MERCK & CO INC ;JANSEN KATHRIN U (US); COOK JAMES C III (US); GEOR) 23 November 1995 (1995-11-23) example 39 -----	1,12,13, 18,22
Y	-----	14-16, 19,20, 23,24
	-----	-/-

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

21 August 2000

Date of mailing of the international search report

28/08/2000

Name and mailing address of the ISA
 European Patent Office, P.B. 5818 Patentaan 2
 NL - 2280 HV Rijswijk
 Tel. (+31-70) 340-2040, Tx. 31 651 epo nl.
 Fax: (+31-70) 340-3016

Authorized officer

Noë, V

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/02463

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 98 34594 A (MERCK & CO INC ; NG ASSUNTA S (US); HENNESSEY JOHN P (US); MANCINEL) 13 August 1998 (1998-08-13) abstract; claim 1; example 2 page 2, line 24 - line 29 -----	5-7, 10, 11
Y	WO 95 10605 A (UNIV LEEDS INNOVATIONS LTD ; GIBSON TIMOTHY DAVID (GB); PIERCE BARR) 20 April 1995 (1995-04-20) page 1, line 1 - line 4; claims 9,11; table 20 -----	14-16, 23,24

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 00/02463

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO 9844944	A 15-10-1998	AU 6953398	A	30-10-1998
		EP 0973546	A	26-01-2000
		NO 994879	A	07-12-1999
		ZA 9802950	A	19-10-1998
WO 9531532	A 23-11-1995	AU 693203	B	25-06-1998
		AU 2549595	A	05-12-1995
		BG 100974	A	30-04-1998
		BR 9507657	A	09-09-1997
		CA 2189882	A	23-11-1995
		CN 1152935	A	25-06-1997
		CZ 9603366	A	16-07-1997
		EP 0757717	A	12-02-1997
		FI 964592	A	15-11-1996
		HU 76354	A	28-08-1997
		JP 10500847	T	27-01-1998
		NO 964862	A	16-01-1997
		NZ 285941	A	27-05-1998
		PL 317234	A	17-03-1997
		SK 147696	A	06-08-1997
		US 5888516	A	30-03-1999
WO 9834594	A 13-08-1998	EP 0971696	A	19-01-2000
WO 9510605	A 20-04-1995	AU 7817094	A	04-05-1995
		EP 0722492	A	24-07-1996